

## RESEARCH

## Open Access



# Genetic variants of numb gene were associated with elevated total cholesterol level and low density lipoprotein cholesterol level in Chinese subjects, in Xinjiang, China

Mayila Abudoukelimu<sup>1,2,4,5†</sup>, Zhen-Yan Fu<sup>1,2,4,5†</sup>, Yang Xiang<sup>1,2,4,5</sup>, Yi-Tong Ma<sup>1,2,4,5\*</sup>, Qing Zhu<sup>1,2,4,5</sup>, Minawaer Abudu<sup>3,4</sup>, Dilare Adi<sup>1,2,4,5</sup>, Yi-Ning Yang<sup>1,2,4,5</sup>, Xiao-Mei Li<sup>1,2,4,5</sup>, Xiang Xie<sup>1,2,4,5</sup>, Fen Liu<sup>2,5</sup> and Bang-Dang Chen<sup>2,5</sup>

## Abstract

**Background:** Hypercholesterolemia is one of the most common risk factors for Coronary Artery Disease (CAD), which is the leading cause of death worldwide. As Numb is an important regulating factor regarding intestinal cholesterol absorption and plasma cholesterol level, the aim of the present study is to investigate the relationship between human Numb gene polymorphism and cholesterol level in Chinese subjects.

**Methods:** All participants came from the First Affiliated Hospital of Xinjiang Medical University (Male: 1052 and Female: 596), and four tagging SNPs (rs2108552, rs12435797, rs1019075 and rs17781919) of Numb gene were genotyped by using TaqMan<sup>®</sup> assays and analyzed in an ABI 7900HT Fast Real-Time PCR System. Further, general liner model was applied for assessing the relationship between cholesterol level and genotypes.

**Results:** By analyzing a dominant model, recessive model and an additive model, we have found that SNP rs2108552 was associated with total cholesterol (TC) and low density lipoprotein-cholesterol level (LDL-C) ( $P = 0.000$  and  $P = 0.007$ ;  $P = 0.042$  and  $P = 0.009$ ;  $P = 0.006$  and  $P = 0.030$ ). C allele of SNP rs17781919 had significantly lower plasma TC level ( $3.46 \pm 0.74$  mmol/L vs  $4.27 \pm 1.1$  mmol/L) and LDL-C level ( $0.98 \pm 0.55$  mmol/L vs  $2.64 \pm 0.93$  mmol/L) when compared with T allele. Additionally, SNP rs12435797 was associated with TC level and SNP rs1019075 was associated with LDL-C level by analyses of a dominant model, recessive model and an additive model ( $P = 0.000$ ,  $P = 0.005$  and  $P = 0.004$ ;  $P = 0.016$ ,  $P = 0.008$  and  $P = 0.033$ ). Further, the association of rs2108552, rs12435797, rs1019075 and rs17781919 with aforementioned different kinds of cholesterol levels remained statistically significant after multivariate adjustment of ethnicity, gender, age, smoking and obesity.

**Conclusions:** Our results indicated that both rs2108552 and rs17781919 in the Numb gene were associated with total cholesterol level and density lipoprotein-cholesterol level in Chinese subjects.

\* Correspondence: [myt\\_xj@sina.com](mailto:myt_xj@sina.com)

<sup>†</sup>Equal contributors

<sup>1</sup>Department of Cardiology, the First Affiliated Hospital of Xinjiang Medical University, Urumqi 830001, People's Republic of China

<sup>2</sup>Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi 830001, People's Republic of China

Full list of author information is available at the end of the article



© 2015 Abudoukelimu et al. **Open Access** This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

## Background

Coronary artery disease (CAD) is the leading cause of morbidity and mortality in developed countries, and becoming increasingly prevalent in developing countries [1–3]. Regarding Coronary artery disease, strong evidence indicates that the risk increase of CAD [4–7] is closely related to the rise of plasma cholesterol level.

Studies have demonstrated that a reduction in plasma total cholesterol (TC) and triglycerides (TG) concentration independently reduces the risk of CAD [8, 9]. In addition, convincing data also shows that 1 % decrease in low density lipoprotein-cholesterol (LDL-C) concentration reduces the risk of CAD for approximately 1 % [10], and 1 % increase in high density lipoprotein-cholesterol (HDL-C) concentration reduces the risk of CAD for approximately 2 % [11]. Further, other than being determined by environmental factors, the levels of plasma cholesterol are also influenced by the genetic constitution of each individual, such as single nucleotide polymorphisms (SNPs) [12–15].

Numb is a member of clathrin-associated sorting proteins, and combines with several other endocytic proteins [16–19]. Pei-Shan Li et al. [20] has revealed that Numb is an essential regulating factor for maintaining human cholesterol homeostasis. Numb regulates cholesterol absorption, and thus plays a crucial role in the development of atherosclerosis and indicated that pharmacologically targeting the Numb-NPC1L1 interaction could be a way to decrease cholesterol absorption and cholesterol level. Further, Jian Wei et al. [21] has confirmed that there does exist a remarkable correlation between Numb polymorphism G595D (rs17781919), a nonsynonymous variant in the coding region of Numb gene- and low concentration of LDL-C among humans. They demonstrated that rs17781919 influences Numb activity of driving NPC1L1 internalization and decreases cholesterol absorption and leading to low plasma cholesterol level.

Unfortunately, the relationship between human Numb gene and lipid profile has not been thoroughly studied yet. Therefore, the aim of the present study is to reveal the relationship between some genetic variants of human Numb gene and lipid profile among Chinese population in Xinjiang, China, hoping to provide additional information to characterize the genetic factors that influence plasma cholesterol levels.

## Methods

### Subjects

Altogether, 1742 (Male:  $n = 1117$ ; Female:  $n = 625$ ) were randomly selected from the First Affiliated Hospital of Xinjiang Medical University that belong to a time period ranges from January 2007 to December 2013 for DNA analysis. The inclusion and exclusion criteria was such as: The analysis presented in this study was based on

1648 subjects (Male:  $n = 1052$ ; Female:  $n = 596$ ) who had passed the eligibility criteria and had complete data on Numb genotype. Further, all of these subjects live in Xinjiang Uighur Autonomous Region of China, and none of them suffers from impaired malignancy, connective tissue disease, renal function, valvular disease or chronic inflammatory disease. Moreover, subjects also are free from thyroid disease, or any history of taking lipid-lowering drugs. In addition we excluded 92 hypercholesterolemia (fasting plasma TC  $\geq 6.26$  mmol/L or fasting plasma LDL-C  $\geq 4.14$  mmol/L [22]) patients during the analysis.

### Lifestyle measurements and blood biochemical analysis

Height and body weight were measured as described previously [23], and body mass index (BMI) was calculated by dividing the weight in kilogram to the square of height in meter. The status of smoking was described as smokers (including current and ex-smokers) or non-smokers. Further, WHO Asia-Pacific Area criterion- BMI  $\geq 25$  kg/m<sup>2</sup> was used to define obesity as described in detail previously [23]. Finally, blood urea nitrogen (BUN), creatinine (Cr), uric acid, glucose, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C), and high density lipoprotein-cholesterol (HDL-C) were measured by using chemical analysis equipment (Dimension AR/AVL Clinical Chemistry System, Newark, NJ) in Clinical Laboratory Department of the First Affiliated Hospital of Xinjiang Medical University [24, 25].

### Ethical approval of the study protocol

All participants have given their written informed consent and explicit permission for DNA analysis as well as for the collection of relevant clinical data. This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinjiang Medical University (Urumqi, China) and was conducted by strictly following the requirements of the Declaration of Helsinki.

### SNP selection

The human Numb gene includes phosphotyrosine-binding (PTB) domain and proline-rich region (PRR) domain [26–29] and has four transcript variants that encode four different isoforms, which are p72 (PTBLPRRL), p71 (PTBSPRRL), p65 (PTBSPRRS), and p66 (PTBLPRRS). Isoform p72 of the Numb gene, which has the longest length among the four isoforms, consists of 651 amino acids. It is located on chromosome 14q24.3 and contains 13 exons which are further separated by 12 introns. There are 3781 different kinds of SNPs of human Numb gene as listed in the National Center for Biotechnology Information SNP database (<http://www.ncbi.nlm.nih.gov/SNP>). For the current study, we have screened the HapMap phase I & II

database and Haploview 4.0 software for the tag SNPs of Numb gene and selected three SNPs (rs2108552, rs12435797, and rs1019075). Meanwhile, we also included rs17781919 from the Numb gene which was associated with LDL-C [21]. SNPs with relatively high minor allele frequencies (MAFs) have been shown to be useful as genetic markers in genetic association studies [30] and all four tag SNPs that selected for the present study had a MAF of  $\geq 0.1$ . In addition, linkage disequilibrium (LD) is the non-random association of alleles at different loci, and we described LD patterns with  $r^2 \geq 0.8$  as a cut-off for the tag SNPs [31].

### Genotyping

Blood samples were taken from all participants by using anticoagulant ethylene diaminetetraacetic acid (EDTA) tube, and standard phenol-chloroform method was used to extract genomic DNA from peripheral leukocytes [32]. Further, genotyping was undertaken by using TaqMan<sup>®</sup> assays from Applied Biosystems following the manufacturer's suggestions and analyzed in an ABI 7900HT Fast Real-Time PCR System [33], and the detailed information of the TaqMan SNP Genotyping method was as follows. Allele-specific fluorogenic probes were hybridised to the template in the first step of the 5' nuclease assay. Then, during the polymerase chain reaction (PCR), the 5' nuclease activity of the Taq polymerase made it possible for discrimination. In addition, the probes include a 3' minor groove binding group that hybridises to single-stranded targets and has greater sequence specificity when compared to the original DNA probes. This reduces nonspecific probe hybridization which leads to low background fluorescence for the 5' nuclease PCR assay

(TaqMan; Applied Biosystems). Cleavage results in the increased emission of a reporter dye. Two unlabeled PCR primers and two allele-specific probes were required for each 5' nuclease assay. At the 5' end, each probe is labeled with two reporter dyes. In the present study, VIC and FAM were used as the reporter dyes. The probes and primers used in TaqMan<sup>®</sup> SNP Genotyping Assays (ABI) were selected according to the information on ABI website (<http://www.appliedbiosystems.com/absite/us/en/home.html>). Finally, PCR amplification was performed by using 0.05  $\mu$ L probes, 3  $\mu$ L of TaqMan Universal Master Mix, 1  $\mu$ L DNA, and 1.95 ddH<sub>2</sub>O in a 6  $\mu$ L final reaction volume. In addition, thermal cycling conditions for PCR amplification were 95 °C for 10 min, 45 cycles of 95 °C for 10 s and 60 °C for 1 min. Moreover, thermal cycling was undertaken by using Applied Biosystems 7900HT Standard Real-Time PCR System, and all 96 well plates were read according to Sequence Detection Systems (SDS) automation controller software v2.3 (ABI).

### Quality control

There was at least one negative and one positive control per 96-well DNA plate in our assays. We have found 100 % concordance between the genotyped duplicate samples (10 %) for each of the SNPs by testing the accuracy of the genotyping. The rate of genotyping success for each SNP was 98 %.

### Statistical analysis

All statistical analyses were performed via using SPSS16.0 software for Windows (SPSS Institute, Chicago, IL, USA). Further, Hardy-Weinberg equilibrium was assessed by  $\chi^2$  analysis for all subjects, and all of the

**Table 1** Clinical and metabolic characteristics of subjects

Risk factor	No. (%) or mean (SD)			P value
	Total	Male	Female	
Age (years)	56.33 (9.83)	55.28 (10.09)	58.22 (9.03)	0.000*
BUN (mmol/L)	5.07 (1.82)	5.01 (1.85)	5.20 (1.78)	0.036*
Cr (mmol/L)	71.04 (17.18)	72.26 (17.29)	68.98 (16.79)	0.000*
Uric acid (mmol/L)	313.70 (89.96)	316.76 (87.43)	308.51 (93.79)	0.078
Glucose (mmol/L)	5.68 (2.43)	5.74 (2.41)	5.58 (2.39)	0.189
Total cholesterol (mmol/L)	4.27 (1.11)	4.33 (1.18)	4.23 (1.07)	0.083
Triglyceride (mmol/L)	1.97 (1.49)	2.01 (1.81)	1.94 (1.28)	0.401
LDL-C (mmol/L)	2.65 (0.91)	2.69 (0.99)	2.62 (0.86)	0.188
HDL-C (mmol/L)	1.05 (0.55)	1.04 (0.55)	1.06 (0.54)	0.378
Obesity (%)	1026 (63.1)	641 (61.6)	385 (66.0)	0.077
Smoking (%)	1031 (62.4)	737 (69.9)	293 (49.2)	0.000*

Continuous variables are expressed as mean  $\pm$  SD. Categorical variables are expressed as percentages

The P value of the continuous variables was calculated by the independent samples *t* test. The P value of the categorical variables was calculated by  $\chi^2$  test

BUN blood urea nitrogen, Cr Creatinine, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol

\**P* < 0.05

**Table 2** Distribution of SNPs of Numb gene for study population

Variants		Total N (%)	Male N (%)	Female N (%)	P value
rs2108552 (SNP1)					
Genotype	C/C	281 (17.0)	174 (16.5)	107 (18.0)	0.732
	G/G	568 (34.4)	367 (34.8)	201 (33.7)	
	C/G	799 (48.6)	511 (48.7)	288 (48.3)	
Dominant model	CC	281 (17.0)	174 (16.5)	107 (18.0)	0.464
	CG + GG	1367 (83.0)	878 (83.5)	489 (82.0)	
Recessive model	GG	568 (34.4)	367 (34.8)	201 (33.7)	0.634
	CG + CC	1080 (65.6)	685 (65.2)	395 (66.3)	
Additive model	CG	799 (48.6)	511 (48.7)	288 (48.3)	0.922
	CC + GG	849 (51.4)	541 (51.3)	308 (51.7)	
Allele	C	1361 (41.3)	859 (40.8)	502 (42.1)	0.471
	G	1935 (58.7)	1245 (59.2)	690 (57.9)	
rs12435797 (SNP2)					
Genotype	T/T	532 (32.3)	323 (30.7)	209 (35.1)	0.111
	G/G	348 (21.1)	235 (22.3)	113 (19.0)	
	G/T	768 (46.6)	495 (47.0)	273 (45.9)	
Dominant model	TT	532 (32.3)	323 (30.7)	209 (35.1)	0.063
	GT + GG	1116 (67.7)	730 (69.3)	386 (64.9)	
Recessive model	GG	348 (21.1)	235 (22.3)	113 (19.0)	0.112
	GT + TT	1300 (78.9)	818 (77.7)	482 (81.0)	
Additive model	GT	768 (46.6)	495 (47.0)	273 (45.9)	0.660
	TT + GG	880 (53.4)	558 (53.0)	322 (54.1)	
Allele	T	1832 (55.6)	1141 (54.2)	691 (58.1)	0.031*
	G	1464 (44.4)	965 (45.8)	499 (41.9)	
rs1019075 (SNP3)					
Genotype	C/C	176 (10.7)	115 (10.9)	61 (10.2)	0.846
	T/T	848 (51.5)	536 (51.0)	312 (52.3)	
	C/T	624 (37.9)	401 (38.1)	223 (37.4)	
Dominant model	CC	176 (10.7)	115 (10.9)	61 (10.2)	0.660
	CT + TT	1472 (89.3)	937 (89.1)	535 (89.8)	
Recessive model	TT	848 (51.5)	536 (51.0)	312 (52.3)	0.585
	CT + CC	800 (48.5)	516 (49.0)	284 (47.7)	
Additive model	CT	624 (37.9)	401 (38.1)	223 (37.4)	0.778
	CC + TT	1024 (62.1)	651 (61.9)	373 (62.6)	
Allele	C	976 (29.6)	631 (30.0)	345 (28.9)	0.527
	T	2320 (70.4)	1473 (70.0)	847 (71.1)	
rs17781919 (SNP4)					
Genotype	C/C	1621 (98.4)	1034 (98.0)	587 (99.0)	0.133
	C/T	27 (1.6)	21 (2.0)	6 (1.0)	
Allele	C	3269 (99.2)	2089 (99.0)	1180 (99.5)	0.135
	T	27 (0.8)	21 (1.0)	6 (0.5)	

The P value of genotype was calculated by Fisher's exact test

\*P &lt; 0.05

**Table 3** Associations between rs2108552 and lipid parameters

	Mean $\pm$ SD			Model 1			Model 2		
	Homozygous for wild allele (C)	Heterozygous	Homozygous for rare allele (G)	P (Dom)	P (Rec)	P (Add)	P (Dom)	P (Rec)	P (Add)
TC (mmol/L)	4.35 $\pm$ 1.24	4.34 $\pm$ 1.02	3.89 $\pm$ 1.01	0.000	0.042	0.006	0.000	0.007	0.015
LDL-C (mmol/L)	2.64 $\pm$ 0.96	2.70 $\pm$ 0.90	2.51 $\pm$ 0.83	0.007	0.009	0.030	0.000	0.004	0.011
HDL-C (mmol/L)	1.02 $\pm$ 0.56	1.02 $\pm$ 0.48	1.15 $\pm$ 0.67	0.000	0.213	0.120	0.012	0.913	0.083
TG (mmol/L)	1.99 $\pm$ 1.62	1.97 $\pm$ 1.41	1.91 $\pm$ 1.47	0.478	0.676	0.889	0.386	0.503	0.954

TC total cholesterol, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol, TG triglycerides, Dom dominant model, Rec recessive model, Add additive model

Model 1: Unadjusted model; Model 2: Analysis of covariance adjusted for ethnicity, gender, age, smoking and obesity

continuous variables were expressed via mean  $\pm$  standard deviation. Furthermore, differences among the frequency of obesity, smoking and Numb genotypes were analyzed through  $\chi^2$  test or Fisher's exact test. Finally, after adjusting confounding variables, general linear model analysis was undertaken to test the association between Numb genotypes and lipid profile. In addition,  $P < 0.05$  was considered as a criterion for statistical significance.

## Results

### Characteristics of the subjects

The study cohort includes 1648 subjects (1052 male, 596 female), and all of them were selected from Out-patient and In-patient department of the First Affiliated Hospital of Xinjiang Medical University that belong to a time period ranges from January 2007 to December 2013.

### Results of outcome measures

1. The clinical and metabolic characteristics of the study population are shown separately for male and female in Table 1. In females, plasma concentration of BUN is higher than to males ( $P = 0.036$ ). Further, in males, plasma concentration of creatinine, prevalence of smoking is higher than females (all  $P = 0.000$ ). In addition, there were no significant differences between males and females on plasma concentration of uric acid, glucose, TC, TG, LDL-C and HDL-C or prevalence of obesity (All  $P > 0.05$ ).

2. The distribution of genotypes of SNPs (all genotyped frequencies of SNPs were in Hardy-Weinberg equilibrium, data not shown) of Numb gene is shown in Table 2. We observed that there were no significant differences between males and females for 4 SNPs (All  $P > 0.05$ ; genotypes, dominant, recessive and additive models). In addition, for the alleles, we found that there was significant difference between males and females for SNP rs12435797 ( $P = 0.031$ ). Further, we found that there was no significant difference between males and females for rs2108552, rs1019075, and rs17781919 (All  $P > 0.05$ ).
3. Numb genotypes and lipid profile
  - i. We found that the rs2108552 was significantly associated with plasma TC and LDL-C level in a dominant model, additive model, or recessive model before (All  $P < 0.05$ ) and after multivariate adjustment of ethnicity, gender, age, smoking and obesity (All  $P < 0.05$ ; Table 3). Further, the rs2108552 was significantly associated with plasma HDL-C level in a dominant model before ( $P = 0.000$ ) and after multivariate adjustment ( $P = 0.012$ ; Table 3).
  - ii. By analyzing a dominant model, additive model, or recessive model, we found that the rs12435797 was significantly associated with plasma TC level before (All  $P < 0.05$ ) and after multivariate adjustment of ethnicity, gender, age, smoking and obesity. (All  $P < 0.05$ ; Table 4). In addition, we

**Table 4** Associations between rs12435797 and lipid parameters

	Mean $\pm$ SD			Model 1			Model 2		
	Homozygous for wild allele (T)	Heterozygous	Homozygous for rare allele (G)	P (Dom)	P (Rec)	P (Add)	P (Dom)	P (Rec)	P (Add)
TC (mmol/L)	4.30 $\pm$ 1.28	4.36 $\pm$ 1.09	4.13 $\pm$ 1.02	0.000	0.005	0.004	0.007	0.006	0.016
LDL-C (mmol/L)	2.60 $\pm$ 0.96	2.70 $\pm$ 0.96	2.60 $\pm$ 0.80	0.171	0.279	0.030	0.808	0.545	0.527
HDL-C (mmol/L)	1.03 $\pm$ 0.66	1.01 $\pm$ 0.38	1.11 $\pm$ 0.67	0.001	0.494	0.010	0.874	0.791	0.833
TG (mmol/L)	1.94 $\pm$ 1.35	2.02 $\pm$ 1.60	1.93 $\pm$ 1.43	0.506	0.758	0.381	0.651	0.942	0.545

TC total cholesterol, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol, TG triglycerides, Dom dominant model, Rec recessive model, Add additive model

Model 1: Unadjusted model; Model 2: Analysis of covariance adjusted for ethnicity, gender, age, smoking and obesity



**Table 5** Associations between rs1019075 and lipid parameters

	Mean $\pm$ SD			Model 1			Model 2		
	Homozygous for wild allele (C)	Heterozygous	Homozygous for rare allele (T)	P (Dom)	P (Rec)	P (Add)	P (Dom)	P (Rec)	P (Add)
TC (mmol/L)	4.25 $\pm$ 1.02	4.30 $\pm$ 1.23	4.22 $\pm$ 1.10	0.568	0.553	0.331	0.324	0.120	0.029
LDL-C (mmol/L)	2.68 $\pm$ 0.90	2.63 $\pm$ 0.91	2.57 $\pm$ 0.97	0.016	0.008	0.033	0.029	0.007	0.043
HDL-C (mmol/L)	1.02 $\pm$ 0.77	1.01 $\pm$ 0.46	1.08 $\pm$ 0.55	0.022	0.322	0.047	0.031	0.469	0.062
TG (mmol/L)	1.97 $\pm$ 1.45	1.95 $\pm$ 1.55	2.01 $\pm$ 1.47	0.662	0.936	0.719	0.367	0.707	0.353

TC total cholesterol, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol, TG triglycerides, Dom dominant model, Rec recessive model, Add additive model

Model 1: Unadjusted model; Model 2: Analysis of covariance adjusted for ethnicity, gender, age, smoking and obesity

also found that rs12435797 was associated with HDL-C level in a dominant and an additive model ( $P = 0.001$ ,  $P = 0.010$ ). However, the difference did not remain statistically significant after multivariate adjustment.

- iii. We observed that the rs1019075 was significantly associated with plasma LDL-C and HDL-C level by analyses of a dominant model, additive model, or recessive model before (All  $P < 0.05$ ) and after multivariate adjustment of ethnicity, gender, age, smoking and obesity (All  $P < 0.05$ ; Table 5).
- iv. We found that rs17781919 was significantly associated with TC, LDL-C and HDL-C level (All  $P \leq 0.000$ ) and the difference remained statistically significant after (All  $P \leq 0.001$ ; (Table 6) multivariate adjustment of ethnicity, gender, age, smoking and obesity.
- v. In addition, there exist no significant difference between different genotypes and the alleles of four SNPs in study cohorts for TG level (all  $P > 0.05$ , respectively; Tables 3–6).

## Discussion

### Findings

In this study, we have genotyped four kinds of SNP of Numb gene, and found that variation in Numb gene associates with cholesterol levels among Chinese subjects. This is the first endeavor to study the common allelic

variant in Numb gene and its association with four types of lipid parameters.

Hypercholesterolemia has been observed to be the cause of multiple physiologic outcomes, such as coronary artery disease, diabetes, and obesity [34–36]. Previous study indicates that Numb gene may provide a therapeutic target for hypercholesterolemia [20]. Meanwhile, Numb genetic variant was directly responsible for the decreased LDL-C concentration [21]. However, few researches have been conducted regarding the relationship between polymorphism of Numb gene and lipid profile.

Our study shows that rs2108552 was significantly associated with plasma TC and LDL-C level by analyses of a dominant model, additive model, or recessive model. Such association was remained significant after multivariate adjustment. Further, individuals with the C allele of rs2108552 had significantly higher plasma TG and LDL-C level when compared with G allele. To our knowledge, a high level of LDL-C and TC concentration in plasma increase the risk of atherosclerotic cardiovascular disease. In previous study, we have found that rs2108552 of Numb gene was associated with CAD among Chinese subjects, and thus assumed CC genotype might be a risk genetic marker for CAD. Together, these results might provide convincing evidence for assuming people who carry C allele may have higher probabilities of suffering from atherosclerotic cardiovascular disease (CVD) than people who carry G allele of rs2108552 of Numb gene.

We also observed that C allele of SNP rs17781919 had significantly lower plasma TC level, LDL-C level and high HDL-C level when compared with T allele and further proved that this variation was associated with low level of LDL-C among large population. Moreover, these results also indicate that people who carry C allele reduces the risk of atherosclerotic cardiovascular disease (CVD) when compared to the people who carry T allele.

Similarly, we observed that the rs12435797 was significantly associated with plasma TC level, and rs1019075 was significantly associated with LDL-C and HDL-C level. Further, individuals with GG genotype of rs12435797

**Table 6** Associations between rs17781919 and lipid parameters

	Mean $\pm$ SD		Model 1	Model 2
	Wild allele (T)	Rare allele (C)		
TG (mmol/L)	1.97 $\pm$ 1.50	1.66 $\pm$ 0.93	0.315	0.330
TC (mmol/L)	4.27 $\pm$ 1.11	3.46 $\pm$ 0.74	0.000	0.001
LDL-C (mmol/L)	2.64 $\pm$ 0.93	0.98 $\pm$ 0.55	0.000	0.000
HDL-C (mmol/L)	1.05 $\pm$ 0.49	1.92 $\pm$ 0.49	0.000	0.000

TC total cholesterol, LDL-C low density lipoprotein-cholesterol, HDL-C high density lipoprotein-cholesterol, TG triglycerides

Model 1: Unadjusted model; Model 2: Analysis of covariance adjusted for ethnicity, gender, age, smoking and obesity

demonstrate low TC level compared with individuals with TT genotype, and individuals with TT genotype of rs1019075 also demonstrate low LDL-C level and high HDL-C level compared with individuals with CC genotype. Such association was also remained significant after multivariate adjustment. Therefore, we assumed that people who carry G allele of rs12435797 and T allele of rs1019075 may have lower probabilities of suffering from atherosclerotic cardiovascular disease (CVD) when compared to the people who carry T allele of rs12435797 and C of rs1019075 allele.

In addition, we have not observed a remarkable difference between TG level and three kinds of SNPs of Numb gene. This might indicate Numb gene does not have as strong correlation with TG concentration as it does with cholesterol concentration. Nevertheless, the relationship between TG concentration and Numb gene needs further study.

### Limitations and shortcomings

First, the source of subjects was limited to the First Affiliate Hospital of Xinjiang Medical University, and these subjects may possess some risk factors of cardiovascular disease and different kinds of lifestyles that both might positively influence lipid profile. Second, a longitudinal epidemiological study, over a reasonably long time span, is required to obtain evidence with higher quality and reliability.

### Conclusion

Our results indicate Numb gene rs2108552 and rs17781919 polymorphisms closely associate with cholesterol level, and this kind of association could be a crucial genetic marker and determinant in clinical research. Nevertheless, our study requires further epidemiological survey by resorting to large numbers of samples.

### Abbreviations

CAD: Coronary artery disease; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol; TC: Total cholesterol; TG: Triglycerides; SNPs: Single nucleotide polymorphisms; BMI: Body mass index; BUN: Blood urea nitrogen; Cr: Creatinine; PTB: Phosphotyrosine-binding domain; PRR: Proline-rich region domain; MAF: Minor allele frequency; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; PCR: Polymerase chain reaction; SDS: Sequence detection systems; CVD: Cardiovascular disease.

### Competing interests

The authors declare that they have no competing interests.

### Authors' contributions

MA, ZYF and YTM conceived and designed the study. MA, ZYF, QZ, DA and MA performed the study. YX, XML, XX, FL and BDC analyzed the data. MA, ZYF, YTM and YNY wrote the paper. All authors read and approved the final manuscript.

### Acknowledgements

This work was supported by grants from Science and Technology Program of Xinjiang Uighur Autonomous Region (2013911111).

### Author details

<sup>1</sup>Department of Cardiology, the First Affiliated Hospital of Xinjiang Medical University, Urumqi 830001, People's Republic of China. <sup>2</sup>Xinjiang Key Laboratory of Cardiovascular Disease Research, Urumqi 830001, People's Republic of China. <sup>3</sup>The First Affiliated Hospital of Xinjiang Medical University, Urumqi 830001, People's Republic of China. <sup>4</sup>Present address: Department of Cardiology, the First Affiliated Hospital of Xinjiang Medical University, Li Yu Shan South Road 137, Urumqi 830001, People's Republic of China. <sup>5</sup>Present address: Xinjiang Key Laboratory of Cardiovascular Disease Research, Li Yu Shan South Road 137, Urumqi 830001, People's Republic of China.

Received: 9 June 2015 Accepted: 28 July 2015

Published online: 12 August 2015

### References

- Kreisberg RA, Oberman A. Clinical review 141: lipids and atherosclerosis: lessons learned from randomized controlled trials of lipid lowering and other relevant studies. In *J Clin Endocrinol Metab*. 2002;87(2):423–37.
- Roger VL, Go AS, Lloyd-Jones DM, Adams RJ, Berry JD, Brown TM, et al. Heart disease and stroke statistics-2011 update: a report from the American Heart Association. *Circulation*. 2011;123:e18–209.
- Writing Group M, Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, et al. Heart disease and stroke statistics-2010 update: a report from the American Heart Association. *Circulation*. 2010;121:e46–215.
- Mackay J, Mensah GA. The Atlas of Heart Disease and Stroke (World Health Organization) Geneva; 2004.
- Law MR, Wald NJ, Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *Br Med J*. 2003;326(7404):1423.
- Kuulasmaa K, Tunstall-Pedoe H, Dobson A, Fortmann S, Sans S, Tolonen H, et al. Estimation of contribution of changes in classic risk factors to trends in coronary-event rates across the WHO MONICA Project populations. *Lancet*. 2000;355:675–87.
- Clarke R, Emberson JR, Parish S, Palmer A, Shipley M, Linksted P, et al. Cholesterol fractions and apolipoproteins as risk factors for heart disease mortality in older men. *Arch Intern Med*. 2007;167:1373–8.
- Bhagavan NV. Medical Biochemistry. 4th ed. San Diego: Harcourt/Academic Press; 2002. p. 1016.
- Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Non-fasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. *J Am Med Assoc*. 2007;298:299–308.
- Grundy SM, Cleeman JI, Merz CN, Brewer Jr HB, Clark LT, Hunninghake DB, et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation*. 2004;110:227–39.
- Gotto Jr AM, Brinton EA. Assessing low levels of high-density lipoprotein cholesterol as a risk factor in coronary heart disease: a working group report and update. *J Am Coll Cardiol*. 2004;43:717–24.
- Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, et al. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat Genet*. 2009;41:47–55.
- Chasman DI, Paré G, Zee RY, Parker AN, Cook NR, Buring JE, et al. Genetic loci associated with plasma concentration of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, and apolipoprotein B among 6382 white women in genome-wide analysis with replication. *Circ Cardiovasc Genet*. 2008;1:21–30.
- Edmondson AC, Braund PS, Stylianou IM, Khera AV, Nelson CP, Wolfe ML, et al. Dense genotyping of candidate gene loci identifies variants associated with high-density lipoprotein cholesterol. *Circ Cardiovasc Genet*. 2011;4:145–55.
- Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, et al. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat Genet*. 2009;41:56–65.
- Roncarati R, Sestan N, Scheinfeld MH, Berechid BE, Lopez PA, Meucci O, et al. The gamma-secretase-generated intracellular domain of beta-amyloid precursor protein binds Numb and inhibits Notch signaling. *Proc Natl Acad Sci U S A*. 2002;99:7102–7.
- Dho SE, Jacob S, Wolting CD, French MB, Rohrschneider LR, McGlade CJ. The mammalian numb phosphotyrosine-binding domain. Characterization of binding specificity and identification of a novel PDZ domain-containing numb binding protein, LNX. *J Biol Chem*. 1998;273:9179–87.

18. Nishimura T, Kaibuchi K. Numb controls integrin endocytosis for directional cell migration with aPKC and PAR-3. *Dev Cell*. 2007;13:15–28.
19. Tong X, Zitserman D, Serebriiskii I, Andrade M, Dunbrack R, Roegiers F. Numb independently antagonizes Sanpodo membrane targeting and Notch signaling in *Drosophila* sensory organ precursor cells. *Mol Biol Cell*. 2010;21:802–10.
20. Li PS, Fu ZY, Zhang YY, Zhang JH, Xu CQ, Ma YT, et al. The clathrin adaptor Numb regulates intestinal cholesterol absorption through dynamic interaction with NPC1L1. *Nat Med*. 2014;20:80–6.
21. Wei J, Fu ZY, Li PS, Miao HH, Li BL, Ma YT, et al. The clathrin adaptor proteins ARH, Dab2, and numb play distinct roles in Niemann-Pick C1-Like 1 versus low density lipoprotein receptor-mediated cholesterol uptake. *J Biol Chem*. 2014;289:33689–700.
22. SoRelle R. ATP III, calls for more intensive low-density lipoprotein lowering in target groups. *Circulation*. 2002;106:e9068–8.
23. Patel S, Flyvbjerg A, Koza'kova' M, Frystyk J, Ibrahim IM, Petrie JR, et al. Variation in the ADIPOQ gene promoter is associated with carotid intima media thickness independent of plasma adiponectin levels in healthy subjects. *Eur Heart J*. 2008;29:386–93.
24. Zheng YY, Xie X, Ma YT, Yang YN, Fu ZY, Li XM, et al. Relationship between a novel polymorphism of the C5L2 gene and coronary artery disease. *PLoS One*. 2011;6:e20984.
25. Xie X, Ma YT, Fu ZY, Yang YN, Ma X, Chen BD, et al. Association of polymorphisms of PTGS2 and CYP8A1 with myocardial infarction. *Clin Chem Lab Med*. 2009;47:347–52.
26. Verdi JM, Bashirullah A, Goldhawk DE, Kubu CJ, Jamali M, Meakin SO, et al. Distinct human NUMB isoforms regulate differentiation vs. proliferation in the neuronal lineage. *Proc Natl Acad Sci U S A*. 1999;96:10472–6.
27. Zwahlen C, Li SC, Kay LE, Pawson T, Forman-Kay JD. Multiple modes of peptide recognition by the PTB domain of the cell fate determinant Numb. *EMBO J*. 2000;19:1505–15.
28. Li SC, Zwahlen C, Vincent SJ, McGlade CJ, Kay LE, Pawson T, et al. Structure of a Numb PTB domain-peptide complex suggests a basis for diverse binding specificity. *Nat Struct Biol*. 1998;5:1075–83.
29. Li SC, Songyang Z, Vincent SJ, Zwahlen C, Wiley S, Cantley L, et al. High-affinity binding of the *Drosophila* Numb phosphotyrosine-binding domain to peptides containing a Gly-Pro-(p)Tyr motif. *Proc Natl Acad Sci U S A*. 1997;94:7204–9.
30. Naganuma T, Nakayama T, Sato N, Fu Z, Yamaguchi M, Soma M, et al. Haplotype-based case-control study between human apurinic/apyrimidinic endonuclease 1/redox effector factor-1 gene and cerebral infarction. *Clin Biochem*. 2009;42:1493–9.
31. Li X, Ma YT, Xie X, Yang YN, Ma X, Zheng YY, et al. Association of Egr3 genetic polymorphisms and coronary artery disease in the Uyghur and Han of China. *Lipids Health Dis*. 2014;13:84.
32. Nakayama T, Soma M, Rahmutula D, Ozawa Y, Kanmatsuse K. Isolation of the 5'-flanking region of genes by thermal asymmetric intercalated polymerase chain reaction. *Med Sci Monit*. 2001;7:345–9.
33. Sano M, Kuroi N, Nakayama T, Sato N, Izumi Y, Soma M, et al. The association study of calcitonin-receptor-like receptor gene in essential hypertension. *Am J Hypertens*. 2005;18:403–8.
34. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest*. 2006;116:1793–801.
35. Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat Rev Mol Cell Biol*. 2008;9:367–77.
36. Blüher M. Adipose tissue dysfunction in obesity. *Exp Clin Endocrinol Diabetes*. 2009;117:241–50.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

